Amendment and Response Serial No.: 10/038,984 Confirmation No.: 9705 Filed: January 4, 2002

For: COMPOSITION AND METHOD FOR IN VIVO AND IN VITRO ATTENUATION OF GENE EXPRESSION

USING DOUBLE STRANDED RNA

Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the aboveidentified application:

1-74. (Canceled)

75. (Previously Presented) A method for attenuating the expression of a target gene in a vertebrate cell ex vivo comprising:

explanting a vertebrate cell from a vertebrate organism;

supplying the cell with at least one double stranded RNA in an amount sufficient to specifically attenuate expression of the target gene and not induce interferon- α/β mediated toxicity, wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene in vitro in 400 mM NaCl, 40 mM PIPES ph 6.4, and 1 mM EDTA, at 50°C; and

implanting the cell into a vertebrate organism, wherein expression of the target gene is attenuated in said vertebrate cell.

- 76. (Previously presented) The method of claim 75, wherein the cell is implanted back into the vertebrate organism from which it was explanted.
- (Canceled).
- 78. (Previously presented) The method of claim 75, wherein the double stranded RNA has a length of less than about 200 bases.
- (Previously presented) The method of claim 75, wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least about 25 bases of

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the target gene.

80-81. (Canceled).

- 82. (Previously presented) The method of claim 75, wherein the double stranded RNA is supplied to the cell by delivery to the cell of the double stranded RNA.
- 83. (Previously Presented) The method of claim 82, wherein the double stranded RNA is purified in the absence of phenol or chloroform.
- 84. (Canceled)
- 85. (Previously presented) The method of claim 75, wherein the target gene is an endogenous gene.
- 86. (Previously presented) The method of claim 75, wherein the target gene is a foreign gene.
- 87. (Previously presented) The method of claim 75, wherein the target gene is a chromosomal gene.
- 88. (Previously presented) The method of claim 75, wherein the target gene is an extrachromosomal gene.
- 89. (Previously presented) The method of claim 75, wherein the double stranded RNA is supplied in an amount to completely inhibit expression of the target gene.
- 90. (Previously Presented) The method of claim 75, wherein the double stranded RNA

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comprises a single strand comprising self-complementary portions that are essentially identical to the target gene.

- (Previously presented) The method of claim 75, wherein the double stranded RNA comprises two separate complementary strands.
- 92. (Previously presented) The method of claim 82, wherein the double stranded RNA is treated with RNase prior to delivery to the cell.
- (Previously presented) The method of claim 91, wherein the two strands of the double stranded RNA are annealed in the presence of potassium chloride prior to delivery.
- (Previously presented) The method of claim 75, wherein the function of the target gene is unknown.
- 95. (Previously presented) The method of claim 75, further comprising identifying a phenotypic change in the vertebrate cell associated with attenuated expression of the target gene.
- 96. (Previously presented) The method of claim 75, wherein the target gene is associated with a disease.
- 97. (Previously presented) The method of claim 75, wherein the target gene is associated with a pathogen.
- 98. (Previously Presented) The method of claim 97, wherein the pathogen is selected from the group consisting of a virus, bacterium, fungus or protozoan.
- 99. (Previously Presented) A method for attenuating the expression of a target gene in a vertebrate cell in vitro comprising:

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supplying the cell with at least one double stranded RNA in an amount sufficient to specifically attenuate expression of the target gene and not induce interferon- α/β mediated toxicity, wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene in vitro in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C.

- 100. (Previously Presented) The method of claim 99, wherein the double stranded RNA has a length of less than about 200 bases.
- 101. (Previously Presented) The method of claim 99, wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least about 25 bases of the target gene.
- 102. (Previously Presented) The method of claim 99, wherein the double stranded RNA is supplied to the cell by delivery to the cell of the double stranded RNA.
- 103. (Previously Presented) The method of claim 102, wherein the double stranded RNA is purified in the absence of phenol or chloroform.
- 104. (Previously Presented) The method of claim 99, wherein the target gene is an endogenous gene.
- 105. (Previously Presented) The method of claim 99, wherein the target gene is a foreign gene.
- 106. (Previously Presented) The method of claim 99, wherein the target gene is a chromosomal gene.
- 107. (Previously Presented) The method of claim 99, wherein the target gene is an extrachromosomal gene.

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- 108. (Previously Presented) The method of claim 99, wherein the double stranded RNA is supplied in an amount to completely inhibit expression of the target gene.
- 109. (Previously Presented) The method of claim 99, wherein the double stranded RNA comprises a single strand comprising self-complementary portions that are essentially identical to the target gene.
- 110. (Previously Presented) The method of claim 99, wherein the double stranded RNA comprises two separate complementary strands.
- 111. (Previously Presented) The method of claim 102, wherein the double stranded RNA is treated with RNase prior to delivery to the cell.
- 112. (Previously Presented) The method of claim 110, wherein the two strands of the double stranded RNA are annealed in the presence of potassium chloride prior to delivery.
- 113. (Previously Presented) The method of claim 99, wherein the function of the target gene is unknown.
- 114. (Previously Presented) The method of claim 99, further comprising identifying a phenotypic change in the vertebrate cell associated with attenuated expression of the target gene.
- 115. (Previously Presented) The method of claim 99, wherein the target gene is associated with a disease.
- 116. (Previously Presented) The method of claim 99, wherein the target gene is associated with a pathogen.

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117. (Previously Presented) The method of claim 116, wherein the pathogen is selected from the group consisting of a virus, bacterium, fungus or protozoan.

- 118. (Previously Presented) The method of claim 99, wherein the cell is a zebrafish cell, a murine cell, or a chick cell.
- 119. (Previously Presented) The method of claim 118, wherein the murine cell is a rat cell.
- 120. (Previously Presented) The method of claim 99, wherein the cell is an embryonic cell.
- 121. (New) The method of claim 75, wherein the vertebrate cell is a murine cell.